

SUPPLEMENTAL INFORMATION (Scheiter et al., 2012)

TABLES

SUPPLEMENTAL TABLE 1. Usage of iTRAQ™ reporter groups for quantitative LC-MS/MS analysis. The CD56^{dim/bright} (grey) as well as CD57^{+/-} NK cell subsets (white) were combined 1:1 after iTRAQ™ labeling for MS analysis. MS analysis was done on both NK cell subset combinations, isolated from 5 healthy human blood donors each.

TRAQ™	NK cell subset	n. of obs (D) per peri-
115	CD3-CD56 ^{bright} (CD56 ^{bright})	D1-D5
117	CD3-CD56 ^{dim} (CD56 ^{dim})	
114	CD3-CD56 ^{dim} CD57 ⁺ (CD57 ⁺)	D6-D10
116	CD3-CD56 ^{dim} CD57 ⁻ (CD57 ⁻)	

SUPPLEMENTAL TABLE 2. Cell amounts of sorted NK cell subsets, purities of isolated NK cell subsets and number of identified proteins within single experiments (donors) after LC-MS/MS measurement and protein identification by database searches.

Workflo w A	D1	D2	D3	D4	D5	Workflo w B	D6	D7	D8	D8	D9
Isolated cell amounts											
CD56^{dim} [x10⁷]	2.5	4.5	2.3	1.9	1.2	CD57⁺ [x10⁶]	3	3	3	3	3
CD56^{bright} [x10⁶]	1.8	1.4	1.1	0.9	1.7	CD57⁻ [x10⁶]	3	3	3	3	3
Purities of isolated NK cell subsets											
CD56^{dim} [%]	99.0	99.1	98.9	99.2	98.2	CD57⁺ [%]	97.8	98.5	97.2	99.2	97.1
CD56^{bright} [%]	96.5	96.2	96.9	96.0	97.0	CD57⁻ [%]	99.7	98.5	99.9	99.9	99.5
Number of identified proteins											
CD56^{dim} versus CD56^{bright}	198	168	194	214	171	CD57⁺ versus CD57⁻	237	183	193	263	247
	4	3	8	1	0		1	1	7	8	0

SUPPLEMENTAL TABLE 3. Exclusive protein identifications in NK cell subsets. (A) uniquely identified proteins in CD56^{dim/bright} NK cell analysis and (B) uniquely identified proteins in CD57^{+/-} NK cell analysis, corresponding to the Venn Diagram in Figure 2A. All uniquely identified proteins are indicated with * at Supplemental Table 4 and 5.

A Uniquely identified proteins in CD56^{dim/bright} NK cell subsets

No.	Uniprot Accession	Uniprot Name
1	FHL1_HUMAN	Four and a half LIM domains protein 1
2	SYUA_HUMAN	Alpha-synuclein
3	NUMB_HUMAN	Protein numb homolog
4	KRT82_HUMAN	Keratin, type II cuticular Hb2
5	CCD50_HUMAN	Coiled-coil domain-containing protein 50
6	HBG2_HUMAN	Hemoglobin subunit gamma-2
7	UB2Q2_HUMAN	Ubiquitin-conjugating enzyme E2 Q2
8	HXK3_HUMAN	Hexokinase-3
9	RN123_HUMAN	E3 ubiquitin protein ligase RNF123
10	HIRP3_HUMAN	HIRA-interacting protein 3
11	GTPB6_HUMAN	Putative GTP-binding protein 6
12	CCD86_HUMAN	Coiled-coil domain-containing protein 86
13	UBAP2_HUMAN	Ubiquitin-associated protein 2

B Uniquely identified proteins in CD57^{+/+} NK cell subsets

No.	Uniprot Accession	Uniprot Name
1	PHF1_HUMAN	PHD finger protein 1
2	BRE_HUMAN	BRCA1-A complex subunit BRE
3	CO058_HUMAN	UPF0580 protein C15orf58
4	SMRD1_HUMAN	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1
5	ABHDB_HUMAN	Abhydrolase domain-containing protein 11
6	LS14B_HUMAN	Protein LSM14 homolog B
7	CC134_HUMAN	Coiled-coil domain-containing protein 134
8	ARP19_HUMAN	cAMP-regulated phosphoprotein 19
9	RANB9_HUMAN	Ran-binding protein 9
10	CB047_HUMAN	Uncharacterized protein C2orf47, mitochondrial
11	LACTB_HUMAN	Serine beta-lactamase-like protein LACTB, mitochondrial
12	INO1_HUMAN	Inositol-3-phosphate synthase 1
13	PTN9_HUMAN	Tyrosine-protein phosphatase non-receptor type 9
14	IPO11_HUMAN	Importin-11
15	ATAD1_HUMAN	ATAD1_HUMAN
16	NDUB5_HUMAN	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5, mitochondrial
17	ABC3D_HUMAN	Probable DNA dC->dU-editing enzyme APOBEC-3D
18	SNR27_HUMAN	U4/U6.U5 small nuclear ribonucleoprotein 27 kDa protein
19	RASF4_HUMAN	Ras association domain-containing protein 4
20	VATD_HUMAN	V-type proton ATPase subunit D
21	CP062_HUMAN	UPF0505 protein C16orf62
22	EXOS3_HUMAN	Exosome complex exonuclease RRP40
23	SAMD1_HUMAN	Atherin
24	PIAS1_HUMAN	E3 SUMO-protein ligase PIAS1
25	P52K_HUMAN	52 kDa repressor of the inhibitor of the protein kinase
26	PLCA_HUMAN	1-acyl-sn-glycerol-3-phosphate acyltransferase alpha
27	NCOA7_HUMAN	Nuclear receptor coactivator 7
28	CO044_HUMAN	UPF0464 protein C15orf44
29	ZN574_HUMAN	Zinc finger protein 574
30	SLAP2_HUMAN	Src-like-adaptor 2
31	NEP1_HUMAN	Probable ribosome biogenesis protein NEP1
32	RN167_HUMAN	E3 ubiquitin-protein ligase RNF167
33	S4A7_HUMAN	Sodium bicarbonate cotransporter 3
34	DLG3_HUMAN	Disks large homolog 3
35	GIPC1_HUMAN	PDZ domain-containing protein GIPC1

SUPPLEMENTAL TABLE 4. MS data of the proteome analysis of CD56^{dim} versus CD56^{bright} NK cells, including all identified 2941 proteins. Mascot Protein Score; Number of unique peptides, used for quantification; log₂ regulation factors and Protein Coverage are listed donor specifically (n=5) and summarized as median values.

See attached excel file: SUPPLEMENTAL TABLE3_CD56dimversusCD56bright.xls

SUPPLEMENTAL TABLE 5. MS data of the proteome analysis of CD57⁺ versus CD57⁻ NK cells, including all identified 3224 proteins. Mascot Protein Score; Number of unique peptides, used for quantification; log₂ regulation factors and Protein Coverage are listed donor specifically (n=5) and summarized as median values.

See attached excel file: SUPPLEMENTAL TABLE4_CD57+versusCD57-.xls

SUPPLEMENTAL TABLE 6. Expression patterns of NK cell specific proteins in differently matured CD56^{pos} NK cell subsets. Depicted are log₂ regulation factors (log₂RF), describing the ratio of expression intensity of one protein in CD56^{bright} compared to CD56^{dim} NK cells and between CD57⁺ and CD57⁻ NK cells. Log₂RF median= median of log₂ regulation factors (n=5 donors), n.d. = not detected.

Protein	CD56 ^{bright} log ₂ RF median	CD57 ⁺ log ₂ RF median
Perforin	-1.9	0.4
CD56	1.4	0.0
CD16	-1.0	0.1
LAMP1	-0.5	0.1
LAMP3	0.5	-0.7
Granzyme A	-0.4	-0.4
Granzyme B	-0.7	-0.1
Granzyme H	-1.8	0.3

Granzyme K	1.7	-1.7
Granzyme M	-0.9	-0.4
CXCR4	0.25	-0.3
2B4 (CD244)	n.d.	0
CD2	1.0	-0.4
CD44	1.2	-0.6
DNAM-1 (CD226)	n.d.	0.1
KIR2DS2	n.d.	0.4
KIR3DL2	n.d.	0.35
KIR3DL1	n.d.	0.2

SUPPLEMENTAL TABLE 7. Threshold-determined (+/-0.83 or +/- 0.51, n=3) list of 9 regulated, but strongly deviating (MAD > 0.3) proteins within the CD56^{dim/bright} and CD57^{+/-} NK cell subsets. Included are UniProt accession names and numbers, as well as protein names. The median Mascot Score = median (n=5) value of the peptide Mascot Scores sum per protein, median log₂-RF = median of the log₂-protein regulation factors of 5 approached donors assessed in CD56 (CD56^{dim} compared to CD56^{bright} NK cells) or CD57 (CD57⁺ compared to CD57⁻ NK cells), MAD value – median absolute deviation from the median log₂-regulation factor and Median Protein Coverage in [%]. Functions are transferred from UniProt.

Accession name UniProt	Accession number UniProt	Protein name	Median Mascot score	Log ₂ -RF Median	MAD	Median Protein Coverage [%]	Function	category	Reference
FETU A	P02765	Alpha-2-HS-glycoprotein	265	-1.2	1.4	5.2	Promotes endocytosis, possesses opsonic properties and influences	1	Osawa et al., 2001

				CD56			mineral phase of bone, shows affinity for calcium and barium ions		
CATW	P56202	Cathepsin W	166	-1.1 CD56	1.0	8.8	May have a specific function in the mechanism or regulation of T- and NK cell cytolytic activity, is not essential for cytotoxic capacities in CTLs	4	Brown et al., 1998 Stoeckle et al., 2008
GRAH	P20780	Granzyme H	515	1.8 CD56	0.5	17	Cytotoxic proteinase, promotes cell death in target cells by cytolysis, causes DNA-nicks in target cell	4	Anthony et al., 2010
CD44	P16070	CD44 antigen	276	-0.6 CD57	0.5	7.3	Receptor for hyaluronic acid (HA), mediates adhesion, plays important role in cell migration, involved in lymphocyte activation, recirculation and homing, and in hematopoiesis	2	Sague et al., 2004 Crosby et al., 2009
MK03	P27361	Mitogen-activated protein kinase 3	358.5	-0.8 CD57	0.4	6.25	Signal transduction, mobilizes lytic granules	3	Djeu et al., 2002 Zheng et al., 2008
SH21A	O60880	SH2 domain-containing protein 1A	235	-0.7 CD57	0.4	28	Signal transduction to FYN, inhibitor of SLAM self-association	1	Endt et al., 2007
CD63	P08962	Granulophysin	166	-0.7 CD57	0.4	6.7	Transport-regulating membrane protein, regulates CXCR4	1	Pols et al., 2008
PACN 1	Q9BY11	Protein kinase C and casein kinase substrate in neurons protein 1	206	-1.6 CD56	0.4	11	May play a role in vesicle formation and transport	4	Kessels et al., 2002
CLIC3	O95833	Chloride intracellular channel protein 3	350	0.9 CD56	0.4	25	Can insert into membranes and form chloride ion channels. May participate in cellular growth control	1	Qian et al., 1999

SUPPLEMENTAL TABLE 8. Significance thresholds for determination of conserved protein regulations. The comparison of iTRAQ™-reporter intensities revealed log₂-regulation factors, which describe the abundance of a protein within the assessed NK cell subsets. Stringent statistical evaluation provided significance thresholds to determine robustly regulated proteins among five assessed donors of each NK cell subset combination.

Investigated	m=5	m=4	m=3
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donors (n)			
CD56^{dim} versus CD56^{bright}	0.745	0.809	0.835
CD57⁺ versus CD57⁻	0.467	0.503	0.515

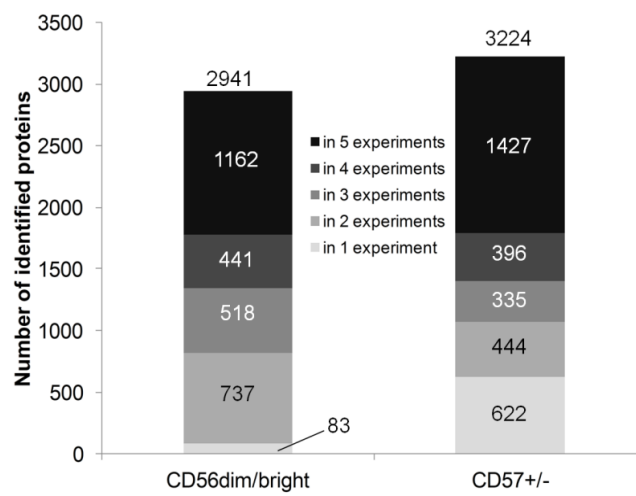
FIGURES

SUPPLEMENTAL FIGURE 1. Number of unique proteins in donor-specific NK cell subsets.

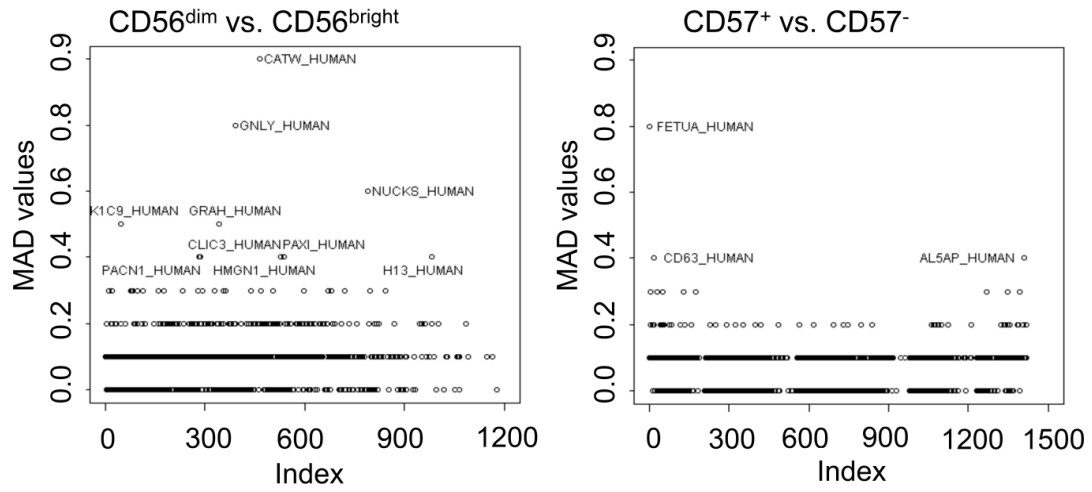
The numbers of proteins are depicted that were detected repeatedly via LC-MS/MS measurement in five analyzed donors per NK subset combination. 40% (1162 of 2941) of all

identified proteins were detected in all five donors of the CD56^{dim/bright} NK cell subsets and 44% (1427 of 3224) proteins within the donor-specific analysis of CD57^{+/-} NK cells.....p.9

SUPPLEMENTAL FIGURE 2. Donor-dependent protein expression in CD56⁺ NK cell subsets. The MAD, median absolute deviation from the median, displays the fluctuations of log₂-regulation factors among the five assessed donors within the CD56^{dim/bright} and CD57^{+/-} NK cell subset datasets, respectively, and gives an overview about proteins that underlie remarkable donor variations within their regulation. By determining the MAD values of proteins, identified in all five investigated human donors of CD56^{dim/bright} or CD57^{+/-} NK cells, donor-dependent protein regulations could be investigated. A MAD value over 0.3 indicates strong deviation. Ten donor-dependently expressed proteins were detected among the CD56^{dim/bright} NK cells subsets and 3 within the CD57^{+/-} NK cell subsets.....p.10



SUPPLEMENTAL FIGURE 1. Number of unique proteins in donor-specific NK cell subsets.



SUPPLEMENTAL FIGURE 2. Donor-dependent protein expression in CD56⁺ NK cell subsets.